PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

OPEN ACCESS

Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2019.241.246

Research Article
The Effect of *Coleus amboinicus* L. Supplementation on *In vitro* Digestibility

[1]Adriani,[2]R. Asra, [3]S. Novianti and [1]Fatati

[1]Department of Animal Production, Faculty of Animal Science, Universitas Jambi, Jambi, Indonesia
[2]Department of Biology, Faculty of Science and Technology, Universitas Jambi, Jambi, Indonesia
[3]Department of Animal Nutrition, Faculty of Animal Science, Universitas Jambi, Jambi, Indonesia

Abstract

Background and Objective: *Coleus amboinicus* L., also known as *bangun-bangun* in Indonesia, is a herb that is often used to increase digestibility and milk production, facilitate metabolic processes in the body and increase VFA. This study aimed to determine the effect of *Coleus amboinicus* L. supplementation on *in vitro* digestibility. **Methodology:** The study used a completely randomized design with 4 treatments and 4 replications: B0 = control (70% forage+30% concentrate), B1 = B0+0.3 g kg^{-1}, B2+0.6 g kg^{-1} and B3 = B0+0.9 g kg^{-1} supplementation with *C. amboinicus*. The observed variables were dry matter digestibility (DMD), organic matter digestibility (OMD), acid detergent fiber (ADF), neutral detergent fiber (NDF), ammonia (NH_3), pH, volatile fatty acid (VFA), acetic acid but yric acid, propionic acid, isobutyric acid, valeric acid, isovaleric acid, bacterial count and rumen protozoa. **Results:** The results demonstrated that supplementation with *C. amboinicus* significantly increased DMD, especially in treatment B1 (74.62±11.30%) and B2 (74.79±6.45%) and increased OMD in B1 (77.74±10.80%) and B2 (78.26±7.76%). However, supplementation with *C. amboinicus* did not affect ADF, NDF, *In vitro* gas production, pH of rumen, valeric acid, isovaleric acid, isobutyric acid, bacterial count or rumen protozoa amount. Supplementation with *C. amboinicus* significantly increased N-NH_3 (33.19±1.54 nM), VFA (106.39±6.20 mM), acetic acid (59.49±1.94 mM), propionic acid (26.50±2.70 mM) and butyric acid (11.49±1.88 mM) in treatment B1. **Conclusion:** Supplementation with *C. amboinicus* may increase DMD, OMD, VFA, acetic acid, propionic acid and butyric acid but does not affect ADF, NDF, pH, gas production, isobutyric acid, valeric acid, isovaleric acid, bacterial count or rumen protozoa.

Key words: *Coleus amboinicus*, *in vitro* digestibility, pH rumen, rumen bacteria, volatile fatty acid

Received: July 25, 2018 Accepted: October 30, 2018 Published: February 15, 2019

Citation: Adriani, R. Asra, S. Novianti and Fatati, 2019. The effect of *Coleus amboinicus* L. supplementation on *In vitro* digestibility. Pak. J. Nutr., 18: 241-246.

Corresponding Author: R. Asra, Department of Biology, Faculty of Science and Technology, Universitas Jambi, Indonesia Tel. +628136648469

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In vitro measures of digestion are conducted by mimicking the processes that occur in the rumen via a fermentor tube. This is a description of the digestibility of feed by rumen microbes that can be done in the laboratory. For the digestion process to take place, it is necessary to note the quality and quantity of the feed[1,2]. One of the plants that could potentially be used as a supplement to improve digestibility and production is *Coleus amboinicus* L *(C. amboinicus*).

The local name of this plant in Indonesia is *torbangun* or *bangun-bangun* and it is a herb that is often used in the North Sumatra Province, Indonesia. The benefit of this plant is that it increases milk production and restores the uterus after delivery. According to Rumetor *et al.*[3] the leaves of *C. amboinicus* can improve the metabolic processes in the body, increase the VFA from 111.15-167.10 mM in feeds with 9%. VFA is the final product of carbohydrate fermentation and is the main energy source of ruminants of rumen origin[4,5]. The production of VFA in the rumen becomes the benchmark of feed fermentability process in ruminants.

Coleus amboinicus has the potential to exhibit antioxidant, diuretic, immunostimulant, anti-inflammatory and anti-infertility effects[6-9]. *Coleus amboinicus* can increase feed consumption, growth and efficiency of dietary use in pigs[10] and can increase reproductive performance[11]. *Coleus amboinicus* defends against worm infection because *C. amboinicus* contains a volatile oil, which can act as an antiseptic[12,13], thereby improving the digestion process. According to Duke[14], *C. amboinicus* contains carvacrol, thymol and forskolin. *Coleus amboinicus* has high content of Fe, carotenoids and potassium to help create a sense of calm, cleanse the blood and relieve pain. The calm conditions of the cattle would optimize the cattle production.

Coleus amboinicus leaves contain flavonoids, saponins and polyphenols[7]. They may increase the hormones prolactin and oxytocin[6]. Lactogogum is a component that can stimulate and increase milk production. For rats, it could increase milk production by approximately 30%[15]. In goats, it could increase milk production by approximately 8.65-67.22%[16], increase dairy goat milk production to 9 g kg^{-1} body weight[17] and restore stamina[18]. Increased production is closely related to the digestive process in the rumen.

Thus, we can determine the effect of *C. amboinicus* on *In vitro* digestibility. An increase in the digestibility of *C. amboinicus* is expected to increase nutrient absorption into cells, thereby increasing milk production. Based on these conditions, it is necessary to do a study to determine the effect of *Coleus amboinicus* L. supplementation on *In vitro* digestibility.

MATERIALS AND METHODS

Coleus amboinicus L. is obtained by harvesting stems and leaves and then chopping them to a size of ±1 cm. The leaves that have been chopped are dried in sunlight for about ±2 days. After the leaves are dried, the leaves are smoothed into flour and then mixed into the concentrate.

This research was conducted *In vitro* by compiling ration for *In vitro* digestibility of 70% elephant grass (*Pennisetum purpureum*) and 30% concentrate (15% bran, 2.5% palm kernel meal, 2.5% coconut meal, 8% soybean meal, 0.5% lime, 0.5% salt, 0.5% urea and 0.5% topmix). All ingredients are mashed and mixed according to the treatment condition. The feed quality can be seen in Table 1.

A completely randomized design was used in this study with 4 treatments and 4 replications: B0 = control (without *C. amboinicus*), B1 = 0.3 g kg^{-1}, B2 = 0.6 g kg^{-1} and B3 = 0.9 g kg^{-1} supplementation with *C. amboinicus*.

Fume rumen fluid fistula was obtained from the Fapet Farm Faculty of Animal Husbandry, University of Jambi and transported to the laboratory in a Thermos. The rumen fluid obtained was squeezed and filtered using gauze and then, the rumen liquid was immediately used for *In vitro* analysis. The rumen liquid was then mixed with a solution of McDougle 1:4. Then, a maximum of 40 mL was inserted into the fermentor tube that was already present with the feed sample. Then, the sample was incubated for 48 h. After 48 h, the tube was opened and microbial activity was stopped by introducing 1 drop of $HgCl_2$ fluid. Then, the fluid was centrifuged at a rate

Table 1: Quality of research feed

	Treatments			
Nutrition of feed (%)	B0	B1	B2	B3
Dry matter	62.10	61.43	61.03	62.32
Protein	14.03	14.21	14.02	14.22
Fat	3.05	4.43	4.34	3.99
Crude fiber	27.42	26.56	26.91	26.67
Ash	9.38	8.74	8.74	9.51

Result of Proximate Analysis, Laboratory of Nutrition and Animal Feed, Faculty of Animal Husbandry, University of Jambi of 2000 rpm. The obtained supernatant was dried in an oven at 600°C for 24 h or until a constant weight and then, the supernatant was analyzed.

The observed variables were as follows: *In vitro* dry-matter digestibility (DMD), organic-matter digestibility (OMD) using the Tilley and Terry method[19], ADF digestibility and NDF were measured using the Van Soest method[20], VFA production was measured using the steam distillation method, $N\text{-}NH_3$ was measured using microdiffusion according to Conway[21], the number of bacteria was measured by enumerating live bacterial colonies and the protozoa of cow rumen fluid were examined according to the method of Ogimoto and Imai[22].

An analysis of variance was used to analyze the data and the Duncan multiple range test was used for post hoc analyses[23].

RESULTS AND DISCUSSION

Degradation of Dry Material, Organic Material, ADF and NDF: Data regarding the Average Dry Matter Digestibility, Organic Matter Digestibility, NDF and ADF feed supplemented with *C. amboinicus* are shown in Table 2.

Supplementation with *C. amboinicus* had a very significant effect on DMD (p<0.01). The treatment of B1 and B2 is significantly higher than B0 and B3. B0 is significantly higher than B3. This result is stronger than that of Rumetor *et al.*[16], who observed the basal baseline DMD was 65.24%. When supplemented with Zn-vitamin E, the DMD yield was 68.23%. Krisnan *et al.*[24] observed that DMD was 58.84% when treated using probion and catalytic supplements. Adriani *et al.*[5] observed that DMD was 65.38-81.56%in the treatment of probiotic and *C. amboinicus.*

This condition is suspected due to the influence of the active compound, carvacrol, contained in the leaves. Carvacrol compounds play a role in reducing the speed of deamination of amino acids and protein degradation in the rumen. The reduction of deamination of amino acids results in the breakdown of proteins, resulting in an increase in the amount of absorbed proteins, which affects the digestibility of dry matter and organic matter[1]. According to Garcia *et al.*[25] increased carvacrol administration of 250 and 500 mg L^{-1} could reduce protein degradation from 72.8-51.5%. Therefore, the protein that escapes degradation is higher and can increase the digestibility of organic matter.

Supplementation with *C. amboinicus* had a significant effect on OMD (p<0.01). Treatment B1 and B2 did not differ from each other but they were significantly higher than B0 and B3. Treatment B0 was significantly higher than B3. This result is relatively similar to Rumetor *et al.*[16], who stated that OMD feed supplemented with *C. amboinicus* ranged from 60.30-76.25%. The increase of OMD in the B1 and B2 treatments may be due to the active substance of carvacrol in *C. amboinicus*, which helps the reduction of the deamination process to increase the amino acid, thereby increasing the digestibility of organic matter[1].

Variability analysis showed that supplementation with *C. amboinicus* did not affect ADF and NDF (p>0.05). The average ADF is 40.08±1.54% and the average NDF is 70.18±0.89%. This result is higher than that of Garcia *et al.*[25], who stated that NDF was decreased by 24.2 and 21.1% after supplementation with carvacrol at approximately 250 and 500 mg L^{-1}, respectively. This result is due to *C. amboinicus* improving the conditions that are conducive to the digestive process, thus yielding better ADF and NDF.

***In vitro* digestibility:** Data regarding *In vitro* digestibility, $N\text{-}NH_3$ and pH after supplementation with *C. amboinicus* are shown in Table 3.

Supplementation with *C. amboinicus* significantly affected $N\text{-}NH_3$ *In vitro* (p<0.01). The B0 and B1 treatments had significantly higher $N\text{-}NH_3$ *In vitro* than B2but relatively similar to B3. These results are similar to those of other studies that obtained amino *In vitro* content in sheep0.45 g kg^{-1} carvacrol; in those studies, the ammonia yield decreased from 36.55/100-23.05/100 mL[26]. This result is relatively similar to those of Rumetor *et al.*[3], who found that increased administration of *C. amboinicus* tends to decrease the production of $N\text{-}NH_3$ *In vitro*. This condition is thought to be due to the influence of carvacrol, an active substance contained in *C. amboinicus*[13]. Ammonia is used by microbes as a source of nitrogen in the synthesis of cell proteins and thus, the presence of ammonia is absolutely necessary. The

Table 2: Average dry matter digestibility, organic matter digestibility, ADF and NDF *In vitro* supplemented by *Coleus amboinicus*

Parameters (%)	B0	B1	B2	B3	Average
DMD	72.37 ± 11.31^{B}	74.62 ± 11.30^{A}	74.79 ± 6.45^{A}	67.92 ± 15.03^{C}	72.43±1.18
OMD	75.65 ± 12.71^{B}	77.74 ± 10.80^{A}	78.26 ± 7.76^{A}	72.31 ± 15.03^{C}	75.99±0.14
ADF	48.93±1.43	46.69±1.33	48.68±1.00	47.99±1.76	48.08±1.54
NDF	70.54±1.14	70.54±0,40	70.65±0.85	69.57±0.85	70.18±0.89

Different capitalized superscripts in one line showed significant differences (p<0.01), DMD: Dry matter digestibility, OMD: Digestibility of organic matter, ADF: Acid detergent fiber, NDF: neutral detergent fiber

Table 3: *In vitro* digestibility, N-NH_3 and rumen gas production after supplementation by *Coleus amboinicus*

Parameters	B0	B1	B2	B3	Average
N-NH_3(nM)	35.28±0.53^A	35.19±1.54^A	33.66±0.12^B	34.10±0.88^{AB}	34.73±1.18
VFA total (mM)	105.33±5.15^a	106.39±6.20^a	93.99±2.87^b	99.65±4.67^{ab}	101.50±6.92
Acetic acid (mM)	59.44±2.52^a	59.49±1.94^a	52.22±3.77^c	56.18±1.20^b	56.90±3.88
Propionic acid (mM)	24.65±1.11^{ab}	26.50±2.70^a	22.20±1.39^b	22.93±2.24^b	23.76±2.18
Butyric acid (mM)	9.73±1.34^b	11.49±1.88^a	9.35±0.55^b	9.74±0.91^b	10.09±0.42
Isobutyric acid (mM)	1.98±0.26	2.01±0.41	1.89±0.26	1.99±0.25	1.98±0.15
Valeric acid (mM)	3.24±0.70	3.42±0.89	3.13±0.50	3.28±0.59	3.29±1.09
Isovaleric acid (mM)	5.59±0.46	5.72±0.58	5.20±0.39	5.48±0.33	5.50±0.45
Gas production (mL)	44.50±5.03	41.23±5.25	44.13±4.21	42.59±2.70	43.11±4.98

Different lowercase superscripts in a row show a marked difference (p<0.05). Different capillary superscripts in a row showed significant differences (p<0.01)

N-NH_3 content in the rumen is an illustration of the process of degradation and the process of protein synthesis by microbes[27].

Supplementation with *C. amboinicus* affected VFA content (p<0.05). Treatment B0 did not differ from B1 but did differ from B2 and B3 did not differ from B0, B1 or B2. The highest VFA was observed in treatment B1. This result is relatively similar to those of Noirot and Bayourthe[28], who stated that administering carvacrol at low doses does not affect the rumen VFA but higher doses of carvacrol decrease the VFA yield. This result is similar to that of Garcia *et al.*[25], who observed that the administration of carvacrol at 250 and 500 mg L^{-1} decreased VFA from 17.6-7.87 mmol L^{-1}. This result is due to *C. amboinicus* being antimicrobial; thus, its administration needs to be closely monitored. The use of plant extracts for digestion engineering is carried out in controlled doses[29].

Coleus amboinicus leaves affected the resulting acetic acid concentration (p<0.05). Treatment B0 and B1 differed from B2 and B3 but B0 and B1 did not differ significantly (p>0.05). This finding is similar to the results for *In vitro* VFA, where treatment B1 is higher than other treatments. This result is higher than what Prihartini[28] observed-that rumen acetic acid ranged from 20.01-47.25 mM. Acetic acid describes the digestibility of crude fiber in the rumen.

Coleus amboinicus treatment influenced propionic acid concentration (p<0.05). Treatment B1 was significantly higher than B2 and B3 but similar to B0, while treatment B2, B3 and B1 did not differ (p>0.05). The highest content of propionic acid was found in treatment B1. This result is thought to be due to the presence of other compounds that create a barrier in the form of antimicrobial thymol, such that higher doses of thymol can reduce propionic acid. Castilejos *et al.*[1] found that administration of thymol compounds could decrease the production of propionic acid but do not affect the *In vitro* VFA content.

Coleus amboinicus treatment had a significant effect on butyric acid (p<0.05). Treatment B1 was significantly higher than B0, B2 and B3 (p<0.05) but B0, B2 and B3 did not differ (p>0.05). This result is higher than what Prihartin[30] observed, where butyric acid ranged from 3.46-7.84 mM in feed treated with lignochloritic inoculum. However, this finding is lower than what Koyuncu and Canbolat[26] observed, where butyric acid ranged from 12.23-13.09 mM in the feed supplemented with carvacrol.

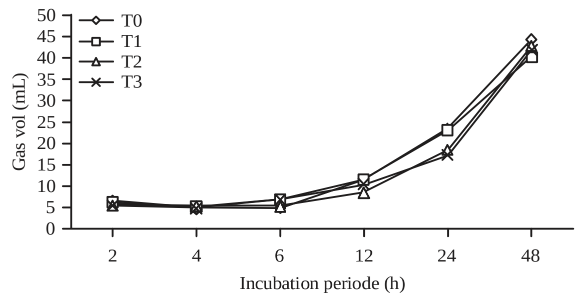

Fig. 1: *In vitro* gas production supplemented with *Coleus amboinicus* leaves

Coleus amboinicus leaves did not affect the content of isobutyric acid, valeric acid or isovaleric acid (p>0.05). The average content of isobutyric acid, valeric acid and isovaleric acid are 1.98±0.15, 3.29±1.09 and 5.50±0.45, respectively. The results of this study are higher than what Xia *et al.*[27] observed, where isobutyric acid ranged from 0.43-0.53 mmol L^{-1}, valeric acid ranged from 0.61-0.86 mmol L^{-1} and isovaleric acid ranged from 0.74-1.02 mmol L^{-1} in rations with molasses.

Coleus amboinicus treatment did not affect gas production for 48 h (p>0.05). Average gas production for 48 h was 43.11±4.98 mL. This result is higher than those of Njidda and Nasiru[31], who observed that *In vitro* gas production ranged from 1.16-25.50 mL per 200 mg DM. The production of gas produced during the *In vitro* process in Fig. 1.

Figure 1 shows that the production of *In vitro* gas produced between the second hour and the sixth hour is relatively constant before increasing at the 12th, 24th and 48th h. This result is due to the action not affecting the activity of rumen microbes, such that the resulting gas yield remains relatively constant.

Table 4: Number of bacteria, protozoa and pH of rumen supplemented with *Coleus amboinicus*

Parameters	B0	B1	B2	B3	Average
pH rumen	6.64±0.11	6.58±0.10	6.63±0.08	6.68±0.07	6.63±0.09
Bacteria (10^8)	8.69±0.09	8.63±0.14	8,64±0.12	8.64±0.22	8.65±0.14
Protozoa (10^6)	1.85±0.41	1.79±0.92	1.81±0.72	1.79±0.55	1.81±0.66

Number of microbes and rumen pH: The number of bacteria, protozoa and pH of rumen supplemented with *C. amboinicus* are shown in Table 4.

Coleus amboinicus treatment did not affect the pH of rumen fluid (p>0.05). The mean pH of rumen fluid is 6.63±0.09. The results are similar to Rumetor *et al.*[16], who stated that *C. amboinicus* supplementation yielded pH rumen of 6.14-6.25, a pH between 5.5-7.0[32] and a pH between 6.76-6.94[24]. The average pH of the normal rumen is 6-7[33], while the ideal pH of the digestome is 6.4-6.8[34]. Suitability of pH rumen can help the process of bacterial colonization on the cell wall and encourage cellulase bacterial activity. If the rumen pH is higher than 7.3, the ammonia absorption process will be accelerated. This condition occurs because the formation of unionized ammonia more easily passes through the rumen wall.

Coleus amboinicus treatment did not affect the amount of rumen bacteria (p>0.05). The rumen bacterial averages were $8.65 \pm 0.45 \times 10^8$. These results were higher than other studies[3]which found that rumen microbials were between 46 -52 x 10^5, 0.8×10^{11} in concentrated 3% BW[35].

Coleus amboinicus treatment did not affect the number of rumen protozoa (p>0.05). The average rumen protozoa is 18.09×10^5. This result is higher than that of Wanapat and Khampa[35], who observed that rumen protozoa fed 3% BW concentrate at 4.1×10^5. Protozoa in the rumen is functioning to digest fermentable carbohydrates and breakdown the hard-to-digest carbohydrates.

The results of this study can improve the digestibility of dry matter, organic matter and VFA. This increase in digestibility will increase the availability of nutrients in the blood, thereby increasing milk production.

CONCLUSION

Coleus amboinicus supplementation may increase DMD, OMD, VFA, acetic acid, propionic acid and butyric acid but has no effect on ADF, NDF, pH, gas production, isobutyric acid, valeric acid, isovaleric acid, bacterial count and rumen protozoa.

ACKNOWLEDGMENTS

We would like to thank the Directorate of Research and Community Service, Directorate General for Research and Development, Ministry of Research, Technology and Higher Education, who funded this activity, according to Research Contract No.22/UN21.17/PP/2017.

REFERENCES

1. Castillejos, L., S. Calsamiglia and A. Ferret, 2006. Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in *in vitro* systems. J. Dairy Sci., 89: 2649-2658.
2. Pineiro-Vazquez, A.T., G. Jimenez-Ferrer, J.A. Alayon-Gamboa, A.J. Chay-Canul, A.J., Ayala-Burgos, C.F. Aguilar-Perez and J.C. Ku-Vera, 2018. Effects of quebracho tannin extract on intake, digestibility, rumen fermentation and methane production in crossbred heifers fed low-quality tropical grass. Trop. Anim. Health Prod., 50: 29-36.
3. Rumetor, S.D., J. Jachja, R. Widjajakusuma, I.G. Permana and I.K. Sutama, 2007. The effect of *Coleus amboinicus* L. leaves and zinc-vitamin E combination in basal diet on the *in vitro* rumen fermentation of Ettawa Crossbred. Proceedings of 6th National Seminar Asosiasi Ahli Nutrisi dan Pakan Indonesia (AINI), July 26-27, 2007, Yokyakarta, Indonesia.
4. McDonald, P., R.A. Edwards, J.F.D. Greenhalgh and C.A. Morgan, 1995. Animal Nutrition. 5th Edn., John Wiley and Sons, Inc., New York.
5. Adriani, R. Azra, S. Novianti and Fatati, 2018. Digestibility of dry and organic material through leaf bangun-bangun (*Coleus amboinicus* Lour) and probiotics supplementation. Proceedings of the National Seminar and Meeting of Agriculture Dean of BKS PTN West Indonesia, July 3-5, 2018, Faculty of Agriculture University of Sultan Ageng Tirtayasa, Banten, Indonesia.
6. Damanik, R., M.L. Wahlqvist and N. Wattanapenpaiboon, 2006. Lactagogue effects of *Torbangun*, a Bataknese traditional cuisine. Asia Pac. J. Clin. Nutr., 15: 267-274.
7. Kaliappan, N.D. and P.K. Viswanathan, 2008. Pharmacognostical studies on the leaves of *Plectranthus amboinicus* (Lour) Spreng. Int. J. Green Pharm., 2: 182-184.
8. Mangathayaru, K., D.V. Pratap, D. Thirumurgan, P.S. Patel, D.J. David and J. Karthikeyan, 2005. Essential oil composition of *Coleus amboinicus* Lour. Indian J. Pharm. Sci., 67: 122-123.
9. Selvakumar, P., 2012. Studies on the antidandruff activity of the essential oil of *Coleus amboinicus* and *Eucalyptus globules*. Asian Pac. J. Trop. Dis., 2: S715-S719.

10. Gunter, K.D. and H. Bossow, 1998. The effect of etheric oil from *Origanum vulgaris* (Ropadiar®) in the feed ration of weaned pigs on their daily feed intake, daily gains and food utilization. Proceedings of the 15th International Pig Veterinary Society Congress, July 5-9, 1998, Birmingham, England.
11. Khajarern, J. and S. Khajarern, 2002. The efficacy of origanum essentiqal oils in sow feed. Int. Pig Topics, Vol. 17.
12. Vasquez, E.A., W. Kraus, A.D. Solsoloy and B.M. Rejesus, 2000. The use of species and medical: Antifungal, antibhacterial, anthelmintic and molluscicidal constituent of Philippine plant. FAO., (Internet). http://www.faoorg/docrep/x2203ow/x2230es
13. Suryowati, T., Rimbawan, R. Damanik, M. Bintang and E. Handharyani, 2015. Antihyperlipidemic activity of torbangun extract (*Celeus amboinicus* Lour) on diabetic rats induced by streptozotocin. IQSR J. Pharm., 5: 50-54.
14. Duke, 2000. Dr. Duke's constituens and ethno-botanical databases. Phytochemical Data-Base, USDA-ARS-NGRL. http://www.ar-sgrin.gov/cgi-bin/duke/farmacy-scroll3.pl.
15. Silitonga, M., 1993. Effects of lactogogum of cumin leaves (*Coleus amboinicu* L. in Lactation mice). Thesis Magiter Saint Graduate Program Biological Studies. Bogor Agricultural University, Bogor, Indonesia.
16. Rumentor, S.D., J. Jachja, R. Widjajakusuma, I.G. Permana and I.K. Sutama, 2008. Supplementation of bangun-bangun leaf (*Coleus amboinicus* Lour) and Zn-vitamin E to improve metabolism and milk production of Etawah cross bred goats. J. Ilmu Ternak Veteriner, 13: 189-196.
17. Jayanegara, A., M. Ridha, E.B. Laconi and Nahrowi, 2014. Katuk and torbangun leaves as galactogogue forages for improving milk yield of dairy goats in Indonesia. Proceedings of the 2nd Asia-Australia Dairy Goat Conference, April 3-6, 2014, Bogor, Indonesia, pp: 1-3.
18. Damanik, R., N. Damanik, Z. Daulay, S. Saragih, R. Premier, N. Wattanapenpaiboon and M.L. Wahlqvist, 2001. Consumption of bangun-bangun leaves (*Coleus amboinicus* Lour) to increase breast milk production among bataknese women in North Sumatera Island, Indonesia. Asia Pac. J. Clin. Nutr., 10: S67-S67.
19. Tilley, J.M.A. and R.A. Terry, 1963. A two-stage technique for the *in vitro* digestion of forage crops. Grass Forage Sci., 18: 104-111.
20. Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. 2nd Edn., Cornell University Press, London, UK., Pages: 476.
21. Conway, E.J., 1950. Microdiffusion Analysis and Volumetric Error. 3rd Edn., Crosby Lokswood and Sons, Ltd., London, Pages: 391.
22. Ogimoto, K. and S. Imai, 1981. Atlas of Rumne Mirobiology. Japan Scientific Societies Press, Jepang.
23. Steel, R.D. and J.H. Torrie, 1982. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Edn., McGraw Hill Book Co., New York, USA.
24. Krisnan, R., B. Haryanto and K.G. Wiryawan, 2009. The effect of combined probiotics with catalyst supplementation on digestion and rumen characteristic in Priangan sheep. Indonesian J. Anim. Vet. Sci., 14: 262-269.
25. Garcia, V., P. Catala-Gregori, J. Madrid, F. Hernandez, M.D. Megias and H.M. Andrade-Montemayor, 2007. Potential of carvacrol to modify *in vitro* rumen fermentation as compared with monensin. Animal, 1: 675-680.
26. Koyuncu, M. and O. Canbolat, 2010. Effect of carvacrol on intake, rumen fermentation, growth performance and carcass characteristics of growing lambs. J. Applied Anim. Res., 38: 245-248.
27. Xia, C., Y. Liang, S. Bai, Y. He, A.U.R. Muhammad, H. Su and B. Cao, 2018. Effects of harvest time and added molasses on nutritional content, ensiling characteristics and *in vitro* degradation of whole crop wheat. Asian-Aust. J. Anim. Sci., 31: 354-362.
28. Noirot, V. and C. Boyourthe, 2005. Effects of carvacrol on the ruminal fermentation *in vitro*. J. Anim. Sci. Vol. 84, Suppl. 1.
29. Calsamiglia, S., M. Busquet, P.W. Cardozo, L. Castillejos and A. Ferret, 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. J. Dairy Sci., 90: 2580-2595.
30. Prihartini, I., S. Chuzaemi and O. Sofjan, 2007. *In vitro* rumen fermentation parameter and gas production rice straw fermented with lignochloritic innoculum. J. Protein, 15: 24-32.
31. Njidda, A.A. and A. Nasiru, 2010. *In vitro* gas production and dry matter digestibility of tannin-containing forges of semi-arid region of North-Eastern Nigeria. Pak. J. Nutr., 9: 60-66.
32. Leng, R.A., 1990. Factor Affecting the Utilization of "Poor Quality" Forage by Ruminants Particulary Under Tropical Condotion. In: Nutrition Research Review, Vol. 3, Smith, R.H. (Ed.). Cambridge University Press, USA.
33. France, J. and R.C. Siddons, 1993. Volatile Fatty Acid Production. In: Quantitative Aspects of Ruminant Digestion and Metabolism, Forbes, J.M. and J. France (Eds.). CAB International, Willingford, UK., pp: 107-122.
34. Erdman, R.A., 1988. Dietary buffering requirements of the lactating dairy cow: A review. J. Dairy Sci., 71: 3246-3266.
35. Wanapat, M. and S. Khampa, 2007. Effect of levels of supplementation of concentrate containing high levels of cassava chip on rumen ecology, microbial N supply and digestibility of nutrients in beef cattle. Asian-Aust. J. Anim. Sci., 20: 75-81.